

# UC Davis

## UC Davis Previously Published Works

### Title

Complete Genome Sequence of Curtobacterium sp. Strain MR\_MD2014, Isolated from Topsoil in Woods Hole, Massachusetts.

### Permalink

<https://escholarship.org/uc/item/7b92f683>

### Journal

Genome announcements, 3(6)

### ISSN

2169-8287

### Authors

Mariita, Richard M  
Bhatnagar, Srijak  
Hanselmann, Kurt  
et al.

### Publication Date

2015-12-01

### DOI

10.1128/genomea.01504-15

Peer reviewed

# Complete Genome Sequence of *Curtobacterium* sp. Strain MR\_MD2014, Isolated from Topsoil in Woods Hole, Massachusetts

Richard M. Mariita,<sup>a</sup> Srijak Bhatnagar,<sup>b</sup> Kurt Hanselmann,<sup>c</sup> Mohammad J. Hossain,<sup>a</sup> Jonas Korfach,<sup>d</sup> Matthew Boitano,<sup>d</sup> Richard J. Roberts,<sup>e</sup> Mark R. Liles,<sup>a</sup> Anthony G. Moss,<sup>a</sup> Jared R. Leadbetter,<sup>f</sup> Dianne K. Newman,<sup>g,h,i</sup> Scott C. Dawson<sup>j</sup>

Department of Biological Sciences, Auburn University, Alabama, USA<sup>a</sup>; Microbiology Graduate Group, University of California Davis, Davis, California, USA<sup>b</sup>; Department of Earth Sciences, ETH Zurich, Zürich, Switzerland<sup>c</sup>; Pacific Biosciences, Menlo Park, California, USA<sup>d</sup>; New England BioLabs, Ipswich, Massachusetts, USA<sup>e</sup>; Linde Center for Global Environmental Science, California Institute of Technology, Pasadena, California, USA<sup>f</sup>; Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, California, USA<sup>g</sup>; Howard Hughes Medical Institute, Pasadena, California, USA<sup>h</sup>; Division of Geological and Planetary Sciences, California Institute of Technology, Pasadena, California, USA<sup>i</sup>; Department of Microbiology and Molecular Genetics, University of California Davis, Davis, California, USA<sup>j</sup>

R.M.M. and S.B. are co-first authors.

**Here, we present the 3,443,800-bp complete genome sequence of *Curtobacterium* sp. strain MR\_MD2014 (phylum *Actinobacteria*). This strain was isolated from soil in Woods Hole, MA, as part of the 2014 Microbial Diversity Summer Program at the Marine Biological Laboratory in Woods Hole, MA.**

Received 30 October 2015 Accepted 4 November 2015 Published 31 December 2015

**Citation** Mariita RM, Bhatnagar S, Hanselmann K, Hossain MJ, Korfach J, Boitano M, Roberts RJ, Liles MR, Moss AG, Leadbetter JR, Newman DK, Dawson SC. 2015. Complete genome sequence of *Curtobacterium* sp. strain MR\_MD2014, isolated from topsoil in Woods Hole, Massachusetts. *Genome Announc* 3(6):e01504-15. doi:10.1128/genomeA.01504-15.

**Copyright** © 2015 Mariita et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 3.0 Unported license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Scott C. Dawson, [scdawson@ucdavis.edu](mailto:scdawson@ucdavis.edu).

Members of the genus *Curtobacterium*, first defined in 1972 by Yamada and Komagata (1), belong to the family *Microbacteriaceae*, within the phylum *Actinobacteria*. Several *Curtobacterium* isolates associate with plants as either pathogens or non-pathogens. Their cells are generally Gram-positive irregularly shaped motile rods. Here, *Curtobacterium* sp. strain MR\_MD2014 was cocultured with *Streptomyces* sp. strain CCM\_MD2014 from the topsoil collected near a rusted fire hydrant in Woods Hole, MA (41°31'44.65"N 70°40'21.5"W) on 7 July 2014, on starch-arginine-tryptophan (SAT) medium using protocols modified from El-Nakeeb and Lechevalier (2). This strain was then isolated from the coculture by selective culture, using antibiotic discs on a modified LB agar. The identity of each of the isolates was verified using the 16S rRNA gene sequence. This strain was cultivated as part of a student-led microbial isolation and sequencing initiative at the 2014 Microbial Diversity Summer Program at the Marine Biological Laboratory in Woods Hole, MA.

From the original coculture, total DNA extraction was carried out by 1 h of lysozyme digestion, followed by use of the Promega Wizard genomic DNA purification kit. The isolated DNA was quantified using the Promega QuantiFluor double-stranded DNA (dsDNA) system and then size selected for a minimum length of 4 kb. The size-selected DNA was sequenced on the Pacific Biosciences RSII sequencing platform with P5C3 chemistry. The sequenced fragments were assembled using HGAP3 on the SMRT Portal (3). The final assembled circular genome had a size of 3,443,800 bp, with 89× sequencing coverage and a G+C composition of 71.95%.

The genome was annotated using NCBI Prokaryotic Genome Annotation Pipeline version 2.8 (rev. 449627) (4, 5). The identified genes were composed of 2,675 coding sequences (CDSs), 4 rRNA operons, 48 tRNAs, and 1 noncoding RNA (ncRNA) gene.

CRISPRFinder (6) identified one definite clustered regularly interspaced short palindromic repeat (CRISPR) and 13 potential CRISPRs in the genome. The PHAST server (7) predicted three prophages, two of which were incomplete, and one of which was of questionable quality. REBASE (8) identified 3 candidate methylase genes and one methylated motif, GG<sup>m6</sup>AGGC, which was found using Pacific Biosciences SMRT Portal analysis. This was unambiguously assigned to the type IIG restriction enzyme Csp2014I (see organism number 14032 on the REBASE website for details).

Phylogenetic analysis of the 16S rRNA gene sequence using SSU-align (version 0.1) (9) and RAXML (version 8.2.3) (10) revealed that strain MR\_MD2014 belongs to the genus *Curtobacterium*, in the family *Microbacteriaceae*. The 16S rRNA gene-based analysis did not provide sufficient data for a refined classification of this strain at the species level. A phylogenetic reconstruction based upon 42 conserved single-copy-marker genes using CheckM (11) identified *Curtobacterium* sp. YR515 as being the most similar to the strain MR\_MD2014 (see <http://dx.doi.org/10.6084/m9.figshare.1574022>). An average nucleotide identity (ANI) calculation using IMG (12) confirmed that the closest available genome to this strain is *Curtobacterium* sp. YR515, with only 85.3% average nucleotide identity across shared genes.

**Nucleotide sequence accession numbers.** The complete nucleotide sequence of this genome is available through GenBank under the accession no. [CP009755](https://www.ncbi.nlm.nih.gov/nuccore/CP009755). The version described here is CP009755.1.

## ACKNOWLEDGMENTS

The isolation and culturing of this organism were carried out as part of the 2014 Microbial Diversity Summer Program at the Marine Biological Laboratory in Woods Hole, MA. The course was supported by

generous grants from the Simons Foundation (grant 309981 to the Marine Biological Laboratory), the Beckman Foundation, and the Howard Hughes Medical Institute. This material is based upon work supported by the National Aeronautics and Space Administration (grant NNA13AA92A), the National Science Foundation (grant MCB-1417876), and the U.S. Department of Energy (grant DE-FG02-85ER13361). R.M.M. was supported by a fellowship from Alabama EPSCoR GRSP, Auburn University's Cell and Molecular Biosciences (CMB) Summer Fellowship, Selman A. Waksman Endowed Scholarship in Microbial Diversity, the Bernard Davis Endowed Scholarship (grant 47802012050), the Auburn Graduate School Travel Award, and the Joseph Kirby Farrington Endowed Fund for Excellence. These funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

Promega Corporation donated the molecular reagents used in this project. The sequencing for this organism was supported and carried out by Pacific Biosciences. We thank George O'Toole, Alison Butler, Emil Ruff, Arpita Bose, Suzanne Kern, Louis Kerr, Kurt Dahlstrom, Alice Michel, and Cristian Salgado for their assistance and advice.

R.J.R. works for New England BioLabs, a company that sells research reagents, including restriction enzymes and DNA methylases, to the scientific community. J.K. and M.B. are full-time employees at Pacific Biosciences, a company that develops single-molecule real-time sequencing technologies, including the sequencing platform used in this project.

This report was prepared as an account of work sponsored by an agency of the U.S. Government. Neither the U.S. Government nor any agency thereof, nor any of their employees, makes any warranty, expressed or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Any reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the U.S. Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the U.S. Government or any agency thereof.

## REFERENCES

1. Yamada K, Komagata K. 1972. Taxonomic studies on coryneform bacteria. V. Classification of coryneform bacteria. *Appl Microbiol* 18: 417–431. <http://dx.doi.org/10.2323/jgam.18.417>.
2. El-Nakeeb MA, Lechevalier HA. 1963. Selective isolation of aerobic actinomycetes. *Appl Microbiol* 11:75–77.
3. Chin C, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <http://dx.doi.org/10.1038/nmeth.2474>.
4. Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity GM, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O. 2008. Toward an Online Repository of Standard Operating Procedures (SOPs) for (Meta) genomic annotation. *Omics J Integr Biol* 12:137–141. <http://dx.doi.org/10.1089/omi.2008.0017>.
5. Tatusova T, DiCuccio MD, Badretdin A, Chetvernin V, Ciufo S, Li W. 2013. Prokaryotic genome annotation pipeline, the NCBI handbook, 2nd ed. National Center for Biotechnology Information, Bethesda, MD.
6. Grissa I, Vergnaud G, Pourcel C. 2007. CRISPRFinder: a Web tool to identify clustered regularly interspaced short palindromic repeats. *Nucleic Acids Res* 35:W52–W57. <http://dx.doi.org/10.1093/nar/gkm360>.
7. Zhou Y, Liang Y, Lynch KH, Dennis JJ, Wishart DS. 2011. PHAST: a fast phage search tool. *Nucleic Acids Res* 39:W347–W352. <http://dx.doi.org/10.1093/nar/gkr485>.
8. Roberts RJ, Vincze T, Posfai J, Macelis D. 2014. REBASE—a database for DNA restriction and modification: enzymes, genes and genomes. *Nucleic Acids Res* 43:D298–D299.
9. Nawrocki EP. 2009. Structural RNA homology search and alignment using covariance models. Ph.D. thesis. Washington University in St. Louis, St. Louis, MO.
10. Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313.
11. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055. <http://dx.doi.org/10.1101/gr.186072.114>.
12. Varghese NJ, Mukherjee S, Ivanova N, Konstantinidis KT, Mavrommatis K, Kyrpides NC, Pati A. 2015. Microbial species delineation using whole genome sequences. *Nucleic Acids Res* 43:6761–6771. <http://dx.doi.org/10.1093/nar/gkv657>.